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IMMUNE EXPULSION OF THE NEMATODE *ASPICULURIS TETRAPTERA* FROM MICE GIVEN PRIMARY AND CHALLENGE INFECTIONS

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Abstract—BEHNKE J. M. 1975. Immune expulsion of the nematode *Aspiculuris tetraptera* from mice given primary and challenge infections. *International Journal for Parasitology* **5**: 511–515. The distribution of larval *Aspiculuris tetraptera* was studied in 4-week-old male and female CFLP mice. Whereas on days 10–12 the larvae were entirely confined to the anterior third of the colon, by day 14 larvae could be found throughout the colon. After day 17 the larvae were again restricted to the anterior colon. This change in distribution was co-incident with a loss of a large proportion of the worm burden, which occurred more consistently in female than in male mice.

The degree of acquired immunity stimulated by various immunizing regimens was assessed by the survival of a challenge infection in experimental and control mice. It was found that a high level of immunity was achieved by exposure to a 19-day primary infection, a 36-day low-level infection and also by three 6-day infections, in each of which the larvae were removed by piperazine treatment immediately after the crypt phase.

INDEX KEY WORDS: *Aspiculuris tetraptera*; distribution; site location; primary immune response; acquired immunity; sex-resistance; host-response.

INTRODUCTION

THE MOUSE pinworm *Aspiculuris tetraptera* is known to have a direct life cycle (Anya, 1966). Larvae emerge from eggs, after ingestion by the host, in the lower intestine and enter the crypts of Lieberkuhn in the mid-colon within 24 h of infection (Anya, 1966; Behnke, 1974). During the 6–7-day-long crypt phase, host–parasite contact is close but although some larvae penetrate the lamina propria, there is little damage to the host epithelium and no host inflammatory response is evident. Seven days after infection the larvae return to the lumen of the colon and emigrate to the anterior colon (preferred site) where they live in between the colonic rugae in close apposition to the host epithelium (Behnke, 1974). The infection becomes patent on day 24 after infection (Anya, 1966).

Chan (1955), in his record of the distribution of *A. tetraptera* in the mouse colon, found some larvae more distal to the preferred site during the third week of infection, but his figures for total worm recovery show no evidence of a worm loss at this time. Stahl (1961), however, noted that female mice lose approximately 50 per cent of their worm burden in the third week of infection. The present paper reports the results of a study in which the distribution of larvae in the mouse colon was examined in the third week of infection and was correlated with the loss of larvae which occurred concurrently. Evidence is presented to show that following exposure to a primary infection with *A. tetraptera* mice acquire an immunity which results in the accelerated expulsion of challenge infections.

MATERIALS AND METHODS

Four-week-old male and female, CFLP strain (Specific pathogen free) mice were used in all experiments. The maintenance and infection of mice has been previously described (Behnke, 1974). Immediately after a mouse was killed by cervical dislocation, the caecum and colon were removed, placed in a Petri-dish and frozen at −18°C. For examination the intestines were thawed and opened under tap-water in Petri-dishes. Worms were then removed and counted under a low-power dissecting microscope. In distribution studies, however, the colon was first ligatured in five places, about 2 cm apart and then stored at −18°C. Before examination the colon was thawed and divided into ten equal sections, each of which was examined separately as described above.

The drug piperazine citrate, given by oral inoculation was found to be 100 per cent effective in removing larvae of all ages. The dose used was 500 mg/kg body weight, given in 0·1 ml of solution. A period of 1–2 days was allowed to elapse after drug treatment before subsequent reinfection with *A. tetraptera*.

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RESULTS

The distribution of larvae in the colon of male and female mice, 10–20 days post infection

Groups of male and female mice were infected with 250 eggs of *A. tetraptera*. Two to four mice of each sex were killed every 24 h from day 10 to day 20 and the distribution of worms in the mouse colon was recorded. The results are shown in Figs. 1–4.

Larvae were first observed posterior to the proximal colon on day 12 when 5–6 per cent of the larvae were recovered from the mid-colon (Figs. 1 & 3). By day 13 more larvae were distal to the preferred site (sections 1–4) and this movement continued until day 16. On day 17 and thereafter larvae could only be recovered from the proximal region of the colon. Figure 2 shows that the mean

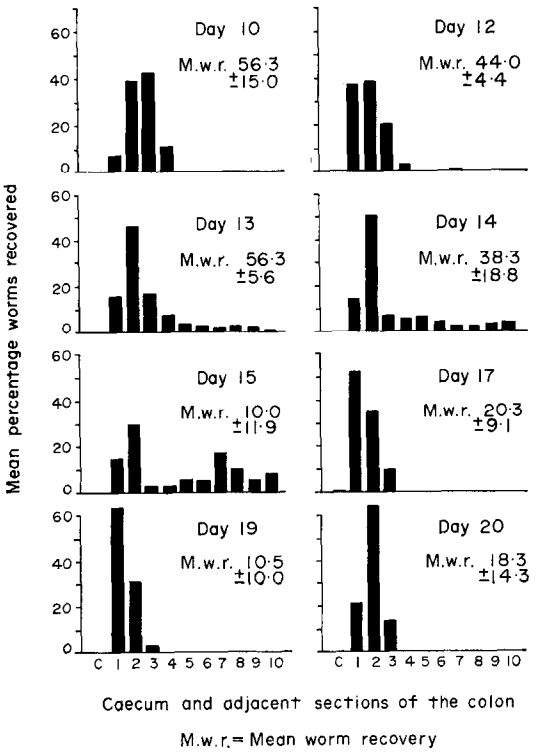

FIG. 1. The distribution of the larvae of *Aspiculuris tetraptera* in the mouse (female) colon after infection with 500 eggs.

worm recovery was reduced by more than 50 per cent in female mice during this time. In some females the entire worm burden was eliminated, whereas in others the residual burden was still comparable to the mean worm recoveries prior to day 14. The loss of worms was greater in female mice than in males since 61·5 per cent (8/13) male mice showed no worm loss whereas worm loss had occurred in all female mice killed after day 15. The

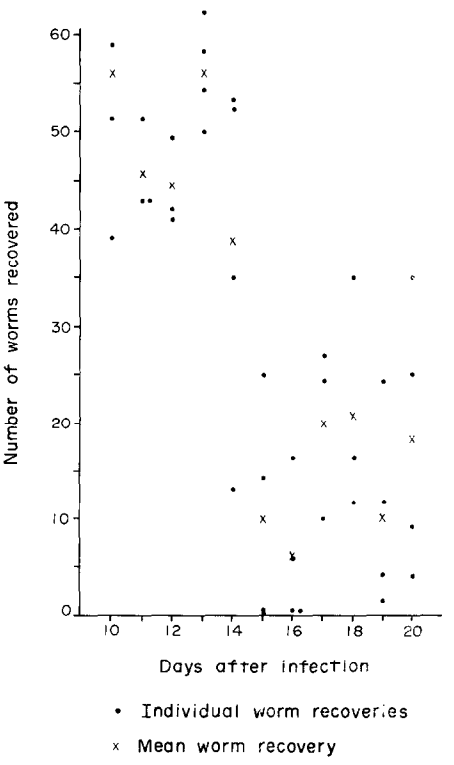

FIG. 2. Mean worm recovery from female mice infected with 500 eggs of *Aspiculuris tetraptera*.

drop in the mean worm recovery (Figs. 3 & 4) of male mice on days 15 and 16 is therefore probably due to the lack of non-resistant mice in these groups.

The stimulation of immunity by a primary infection with A. tetraptera

To ascertain whether a primary infection with *A. tetraptera* would evoke acquired immunity, 3 groups of female mice were given an initial infection of 750 eggs, the course of which was followed by killing one group on day 11 (i.e. before rejection) and another group on day 21 (i.e. after rejection). The remaining group together with a challenge control group, was given piperazine on day 19 and then both groups were challenged with 1200 eggs on day 21. Twelve days after challenge the mice were killed and the worms present were counted. The results are shown in Table 1.

The primary infection was rejected in the normal way. The mean worm burden on day 11 was significantly higher ($P < 0.001$) than that on day 21. Exposure to this infection was, however, sufficient to evoke a high level of immunity to subsequent challenge, as assessed by the difference between the mean numbers of worms recovered from control and previously infected mice.

TABLE 1—THE STIMULATION OF IMMUNITY BY A SINGLE IMMUNIZING INFECTION WITH *A. tetraptera*

Groups	No. of mice	Mean worm recovery	± S.D.
1. Primary infection 750 eggs. Killed day 11	8	137·3†	64·1
2. Primary infection 750 eggs. Killed day 21	11	7·5†	13·9
3. Controls treated piperazine day 19. Primary infection 1200 eggs day 21. Killed day 12	10	108·2*	124·1
4. Primary infection 750 eggs. Piperazine treated day 19. Challenge infection 1200 eggs day 21. Killed day 12	10	0·7*	1·0

Statistical analysis of results: * $0{\cdot}02 > P > 0{\cdot}01$. † $P < 0{\cdot}001$.

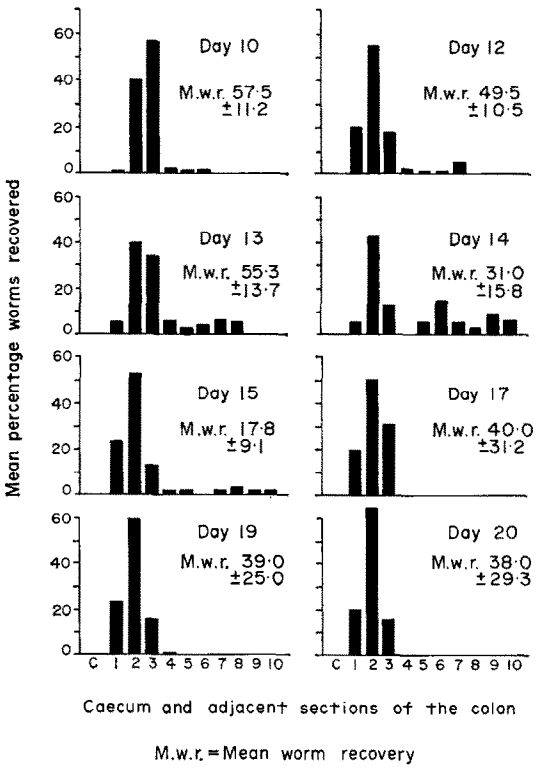

FIG. 3. The distribution of the larvae of *Aspiculuris tetraptera* in the mouse (male) colon after infection with 500 eggs.

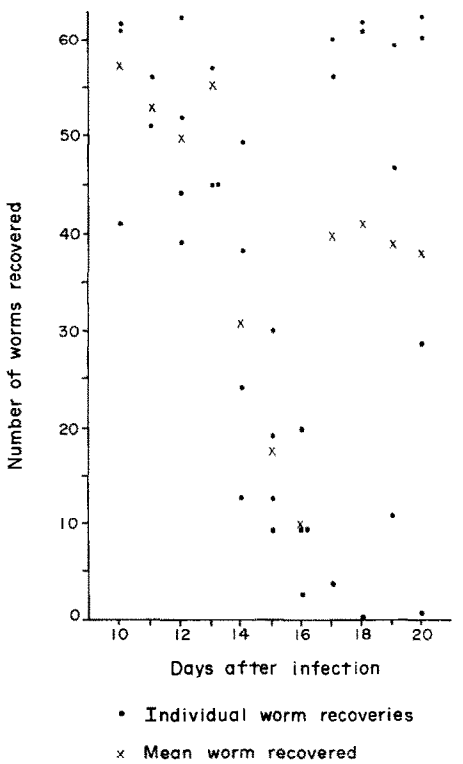

FIG. 4. Mean worm recovery from male mice infected with 500 eggs of *Aspiculuris tetraptera*.

The stimulation of immunity by abbreviated and low-level primary infections

Forty-two female mice were divided into 4 groups. Two groups were infected with 250 eggs on day 0 and one of these groups (group 1) was killed after 10 days. A third group was exposed to 3 infections of 500 eggs, each infection being terminated after 6 days (end of the crypt phase) by piperazine treatment. An interval of one day elapsed between treatment and subsequent reinfection. Group 2, group 3 and a challenge control group (group 4) were treated with piperazine on day 36 and infected with 1000 eggs on day 40. The mice were killed after 11 days and the worms were counted. The results are shown in Table 2.

Three mice in the control group failed to develop a reasonable level of infection following infection with 1000 eggs. Nevertheless, the mean worm recovery in this group was higher than in the remaining groups ($P < 0{\cdot}001$). This result shows that both regimens of primary infection were

TABLE 2—THE STIMULATION OF IMMUNITY BY THE CRYPT PHASE ALONE OR BY A LOW-LEVEL 36-DAY INFECTION WITH *A. tetraptera*

Groups	No. of mice	Mean worm recovery	± S.D.
1. Primary infection 250 eggs. Killed day 10	10	24·9	14·9
2. Primary low-level 36-day infection 250 eggs. Piperazine treated day 36. Challenge infection 1000 eggs day 40. Killed day 11	10	57·6†	82·0
3.* Three crypt phase infections. Piperazine treated day 36. Challenge infection 1000 eggs day 40. Killed day 11	10	10·1‡	17·6
4. Controls treated piperazine day 36. Primary infection 1000 eggs day 40. Killed day 11	12	273·4†‡	254·3

* Group exposed to 3, 6-day infections (crypt phase) each terminated by piperazine treatment before subsequent reinfection.

Statistical analysis of results: † $P < 0{\cdot}001$. ‡ $P < 0{\cdot}001$.

effective in inducing immunity to the challenge infection.

DISCUSSION

The appearance of larval *A. tetraptera* in the mid colon, on day 14 as reported by Chan (1955), was demonstrated here to be part of a major re-distribution of worms in male and female mice taking place during the third week of infection. The movement of up to 80 per cent of the worm burden, from the anterior colon to the rectum, was shown to be co-incident with the period of major worm loss. Although Stahl (1961) noted that female mice lose a proportion of an infection during the third week, he associated this loss with sex resistance, based on the direct detrimental effect of oestrogen on the worms. In the present report it was found that both sexes lost worms during the third week, but the response in female mice was more consistent. The weaker response of some male mice is in agreement with the reports by Mathies (1959) and Stahl (1961). Male sex hormones are known to interfere with the immune response in male animals (Kappas, Jones & Roitt, 1963; Graff, Lappe & Snell, 1969; Blazkovec, Orsini & Maginn, 1973; Castro, 1974) and it is suggested that the weaker response to *A. tetraptera* exhibited by male mice is consequent to the immunodepression resulting from these hormones.

The expulsion of some nematode parasites from the host during the second and third weeks of a primary infection is well documented in the literature (Wakelin, 1967; Denham, 1968; Ogilvie & Jones, 1971, 1973) and it is invariably accompanied by an acquired immunity to further infection manifest by a more rapid expulsion of challenge infections, and a smaller residual worm burden persisting after the events of immunity (Jarrett, Jarrett & Urquhart, 1968; Wakelin, 1973). In order to ascertain whether the expulsion of *A. tetraptera* was mediated by immunological phenomena, it was important to know whether a primary infection initiated a state of acquired immunity. The results of experiments designed to answer this question firmly established that female mice develop an acquired immunity following primary exposure to the parasite. The secondary response was effective within 10–12 days after challenge infection and could be elicited by a 19 day exposure to an infection of 137·3 ± 64·1 larvae or a 36 day exposure to 24·5 ± 14·9 larvae. Furthermore when mice were exposed to the crypt phase of the infection alone, they also acquired immunity to *A. tetraptera* and resisted a challenge infection more effectively than control mice. As Stahl (1966) pointed out, the larvae of *A. tetraptera* in the crypt phase are in close contact with the host epithelium and it is possible that the antigenic stimulus, which evokes the protective immune response, is generated at this time.

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